Symposium: Membrane Trafficking

2467-Symp
Probing the Structure, Topology, and Oligomerization of Caveolin-1
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Caveolae are highly-curved invaginated micro-domains located in the plasma membrane that play a central role in a variety of cellular processes. Caveolins (1, 2, and 3) are the most important proteins found in caveolae, and are responsible for giving caveolae their unusual ''flask-like'' shape. Recent evidence has shown that improper regulation and mutant forms of caveolin can result in a variety of diseases including Alzheimer's, muscular dystrophy, cancer, and heart disease. Caveolin adopts an unusual intramembrane ''horseshoe'' conformation where both its N- and C-termini face the cytoplasm, and this conformation is thought to promote membrane curvature. In addition, via high-order oligomerization, caveolin is thought to form a structural backbone which stabilizes the membrane curvature. Using biophysical techniques (NMR, fluorescence spectroscopy, and analytical ultracentrifugation) we have begun to build a structural and topological model of caveolin-1.

2468-Symp
Pho85/Cdk5 is a Positive Regulator of Phosphatidylinositol 3,5-Bisphosphate via Direct Phosphorylation of Fab1/PIKfyve
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The signaling lipid phosphatidylinositol 3,5-bisphosphate ($PI(3,5)P_2$) plays critical roles in multiple tissues and multiple organisms. $PI(3,5)P_2$ is regulated in part via a protein complex that contains the PI3P-5-kinase Fab1, the $PI(3,5)P_2$ lipid phosphatase, Fig4, and the scaffold Vac14. In yeast, as well as in neurons, $PI(3,5)P_2$ levels are transiently and rapidly elevated in response to selected signals. However to date, the upstream signaling pathways that regulate $PI(3,5)P_2$ have not been identified. Here we report that in the yeast *Saccharomyces cerevisiae*, the CDK, Pho85 and the cyclin, Pho80 are required for the acute elevation of $PI(3,5)P_2$ upon hyper-osmotic shock. Pho85-Pho80 directly phosphorylates Fab1 and positively regulates the synthesis of $PI(3,5)P_2$. Furthermore, we find that Pho85-Pho80 phosphorylation of Fab1 likely generates a conformation change that activates Fab1 lipid kinase activity. Cdk5- p35, the mammalian homologue of Pho85-Pho80, is particularly critical in neuronal physiology and regulate multiple pathways. Notably, we find that mammalian Cdk5-p35 directly phosphorylates peptides of mammalian Fab1/PIKfyve, which strongly suggests that PIKfve is a direct target of Cdk5-p35 in tissue culture cells. These studies reveal a conserved, new down stream target of Cdk5, and provide insights into how $PI(3,5)P_2$ is regulated.

2469-Symp
Dynamin at the Brink of Fission
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Dynamin family members are large GTPases that are associated with diverse cellular processes, including clathrin-mediated endocytosis, fusion and fission of mitochondria, division of chloroplasts and peroxisomes, cell division, and resistance to viral infections. The founding member, dynamin, plays a direct role in endocytosis by assembling around the necks of clathrin-coated pits in a helical array. The self-assembly of dynamin into oligomers stimulates its GTPase activity, which is necessary for efficient fission during endocytosis. Recent evidence suggests that a three-bundle helix, near the G domain, undergoes a dramatic hydrolysis-dependent conformational change that may function as a dynamin powerstroke. To understand how the powerstroke propagates through the helical assembly and contributes to membrane fission, we solved the helical structure of a transition-state-defective dynamin mutant, K44A, using cryo-electron microscopy and image processing techniques. The 3-dimensional map of K44A is a 2-start helix with an inner luminal diameter of 3.7 nm, reaching the theoretical limit for spontaneous fission. Computational docking of G domains with the three bundle helix into the 3D map, reveals that a GTP ground state of dynamin is sufficient to achieve this 'super-constricted' state and shows how the 2-start helical arrangement generates the most efficient packing of dynamin around the membrane neck. Additional structural and biochemical studies are underway to unravel the global conformational changes that are needed to move beyond the final super-constricted pre-fission state.

2470-Symp
Studying Membrane Fusion at the Molecular Level using a Biomimetic Model System
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Fusion of lipid bilayers, or membranes, is a ubiquitous process. It occurs in the cells of our body during cargo exchange by membrane vesicles and during viral infection, but also in industrial processes such as yeast production. While many proteins like SNAREs have been identified that play crucial roles in membrane fusion, the molecular mechanism of fusion remains unclear. This fascinating process is unexpectedly complex and our aim is to understand the details of this process using a biomimetic model system which has all the characteristics of natural membrane fusion; targeted docking, followed by lipid and content mixing in the absence of leakage.
We developed a model system composed of a complementary pair of lipidated peptides able to form a heterodimeric coiled coil motif at the membrane interface similar to natural SNARE subunits. The different steps of membrane fusion are currently studied using biophysical and biochemical techniques with a special focus on peptide-peptide and peptide-lipid interactions. Unravelling of the molecular mechanism of membrane fusion using a multi-faceted approach of complementary techniques will result in a unique, in depth view of the process of membrane fusion.
To date, it is a major challenge to achieve efficient and targeted liposome delivery directly into the cytoplasm of live cells, circumventing endocytotic pathways. In this respect, we recently studied and achieved membrane fusion between liposomes and live cells. Future applications are foreseen in drug delivery, nanoreactors and membrane engineering to name but a few.

Symposium: Nanopores: Methods and Mechanistic Insights

2471-Symp
Polymers through Protein Pores: Single-Molecule Experiments with Nucleic Acids, Polypeptides and Polysaccharides
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When polymers move from one cellular compartment to another, they pass through protein pores. Nucleic acids, polypeptides and polysaccharides are all transported in this way, stimulating questions about the nature of the transported polymer (diameter, stiffness, branching, charge, charge distribution), the driving force (ΔV, ΔpH, refolding, binding) and how that driving force is coupled (direct coupling v diffusion/ ratchet). We have been investigating all three classes of biopolymer by current recording through individual transmembrane pores. We have not only made interesting fundamental discoveries about the translocation processes, but also found useful applications of our work, for example in nucleic acid sequencing and the discovery of antibacterial agents.

2472-Symp
Molybdenum Disulfide Nanopores: Why 3 Atoms are Better than One?
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Atomically thin nanopore membranes are considered to be a promising approach to achieve single base resolution with the ultimate aim of rapid and cheap DNA sequencing. Recently, we made advances in using 2 D materials such as graphene or MoS_2 as nanopore platform .Translocation of various types of DNA exhibits a signal amplitude that is five times higher than in the case of solid-state Si_3N_4 membranes and a SNR of more than 10. These features are highly desirable for event detection and we take advantage of them by showing the electric-field induced unfolding of a 48 kbp long DNA molecule within the nanopore which manifests itself in the quantization of the current drop. Unlike graphene nanopores, no special surface treatment is needed to avoid strong interaction between DNA and the surface. Our results imply that MoS_2 nanopore membranes can compete with graphene nanopore membranes in terms of spatial resolution and possibly better performance for transverse detection.
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